FIG. 10 illustrates the present invention's ability to differentiate spectra using a three-dye mixture of OXAZINE™ 720, 725 and 750. OXAZINE is a trademark of Excition, Inc. We mixed the dyes at equal concentrations of 3.3 μM. We recorded the emission spectra for an excitation signal wavelength of 640 nm and the laser modulation frequency was swept (modulated) from 10 MHz to 140 MHz at 5 MHz increments. The present invention extracted fluorescence lifetimes of 3.705 nsec for Oxazine 720 (196 on FIG. **10**), 1.979 nsec for Oxazine 750 ((198 on FIG. **10**), and 0.5588 nsec for Oxazine 725 (**200** on FIG. **10**). The results from the present invention compared very well with the listed individual dye fluorescence lifetimes of 3.739 nsec for Oxazine 720, 2.014 nsec for Oxazine 750 and 0.9935 nsec for Oxazine 725. The individual spectra extracted for each dye from the mixture revealed spectral characteristics that matched with spectra obtained from the individual dyes.

The present invention overcomes the limitations of the prior art systems by utilizing a novel technique to measure the fluorescence lifetime. Instead of irradiating the target sample with a single short pulse of light, the present invention continuously irradiates the target sample with a light source whose amplitude and phase are modulated with time. This technique allows the present invention to use a chemometric analysis to automatically extract the lifetimes from the 'phase delay' and 'intensity vs. time' characteristics of the emitted light.

The present invention is a system for chemometric analysis for the extraction of the individual fluorescence spectrum and fluorescence lifetime from a target mixture. The present invention comprises a processor with an apparatus for generating an excitation signal to transmit at a target mixture and an apparatus for detecting the emitted signal from the target mixture. The present invention extracts the individual fluorescence spectrum and fluorescence lifetime measurements from the frequency and wavelength data acquired from the emitted signal. The present invention uses an iterative solution that first requires the initialization of several decision variables and the initial approximation determinations of intermediate matrices. The iterative solution compares the decision variables for convergence to see if further approximation determinations are necessary. If the solution converges, the present invention then determines the reduced best fit error for the analysis of the individual fluorescence lifetime and the fluorescence spectrum before extracting the individual fluorescence lifetime and fluorescence spectrum from the emitted signal of the target mixture.

The present invention additionally comprises a method and apparatus for generating and mixing signals for frequency-domain lifetime and spectral fluorometry. The present invention comprises a plurality of signal generators that generate a plurality of signals where the signal generators modulate the amplitude and/or the frequency of the signals. The present invention uses one of these signals to drive an excitation signal that the present invention then directs and transmits at a target mixture, which absorbs the energy from the excitation signal. The property of fluorescence causes the target mixture to emit an emitted signal that the present invention detects with a signal detector. The present invention uses a plurality of mixers to produce a processor reference signal and a data signal. The present invention then uses a processor to compare the processor reference signal with the data signal by analyzing the differences in the phase and the differences in the amplitude between the two signals. The processor then extracts the fluorescence lifetime and fluorescence spectrum of the emitted signal from the phase and amplitude information using a chemometric analysis.

Other embodiments of the invention will be apparent to those skilled in the art after considering this specification or practicing the disclosed invention. The specification and examples above are exemplary only, with the true scope of the invention being indicated by the following claims.

We claim:

1. An apparatus for fluorescence lifetime and spectral measurements, comprising:

a driving/reference signal generator that generates a driving/reference signal, said driving/reference signal is amplitude and/or frequency modulated over time;

a mixing signal generator that generates a mixing signal, said mixing signal is amplitude and/or frequency modulated over time;

an excitation signal generator that generates an excitation signal, the driving/reference signal drives said excitation signal generator;

a signal detector that detects the emitted signal;

a mixer that mixes the emitted signal with the driving/reference signal and produces the processor reference signal;

a mixer that mixes the emitted signal with the mixing signal and produces the data signal; and

a processor that extracts the fluorescence lifetime and fluorescence spectrum of the emitted signal from the comparison of the processor reference signal with the data signal using a chemometric analysis.

2. The apparatus of claim **1** wherein the driving/reference signal and the mixing signal vary by an adjustable offset frequency.

3. The apparatus of claim **1** wherein said chemometric analysis extracts the fluorescence lifetime of the emitted signal from the phase difference between the processor reference signal and the data signal.

4. The apparatus of claim **1** wherein said chemometric analysis extracts the fluorescence spectrum of the emitted signal from the amplitude difference between the processor reference signal and the data signal.

5. The apparatus of claim **1** wherein said chemometric analysis further comprises a converging iterative solution.

6. A system for fluorescence lifetime and spectral measurements, comprising:

means for generating a driving/reference signal, said driving/reference signal means modulates the amplitude and/or the frequency of the driving/reference signal over time;

means for generating a mixing signal, said mixing signal means modulates the amplitude and/or the frequency of the mixing signal over time,

means for generating an excitation signal, the driving/reference signal drives said excitation signal means;

means for detecting the emitted signal;

means for mixing the emitted signal with the driving/reference signal to produce the processor reference signal;

means for mixing the emitted signal with the mixing signal to produce the data signal; and

a processor that extracts the fluorescence lifetime and fluorescence spectrum of the emitted signal from the comparison of the processor reference signal with the data signal using a chemometric analysis.

7. The system of claim **6** wherein the driving/reference signal and the mixing signal vary by an adjustable offset frequency.

8. The system of claim **6** wherein said chemometric analysis extracts the fluorescence lifetime of the emitted

signal from the phase difference between the processor reference signal and the data signal.

9. The system of claim **6** wherein said chemometric analysis extracts the fluorescence spectrum of the emitted signal from the amplitude difference between the processor reference signal and the data signal.

10. The system of claim **6** wherein said chemometric analysis further comprises a converging iterative solution.

11. A method for measuring the fluorescence lifetime and the fluorescence spectrum, comprising the following steps:

generating a driving/reference signal and modulating the amplitude and/or the frequency of the driving/reference signal over time;

generating a mixing signal and modulating the amplitude and/or the frequency of the mixing signal over time;

generating an excitation signal from the driving/reference signal;

detecting the emitted signal,

mixing the emitted signal with the driving/reference signal and producing the processor reference signal;

mixing the emitted signal with the mixing signal producing the data signal; and

extracting the fluorescence lifetime and fluorescence spectrum of the emitted signal from the comparison of the processor reference signal with the data signal to measure using a chemometric analysis.

12. The method of claim **11** wherein the driving/reference signal and the mixing signal vary by an adjustable offset frequency.

13. The method of claim **11** wherein said chemometric analysis extracts the fluorescence lifetime of the emitted signal from the phase difference between the processor reference signal and the data signal.

14. The method of claim **11** wherein said chemometric analysis extracts the fluorescence spectrum of the emitted signal from the amplitude difference between the processor reference signal and the data signal.

15. The method of claim **11** wherein said chemometric analysis further comprises a converging iterative solution.

16. A method of producing an apparatus for fluorescence lifetime and spectral measurements, comprising:

providing a driving/reference signal generator that generates a driving/reference signal, said driving/reference signal is amplitude and/or frequency modulated over time;

providing a mixing signal generator that generates a mixing signal, said mixing signal is amplitude and/or frequency modulated over time;

coupling an excitation signal generator that generates an excitation signal and a reference signal to said driving/reference generator;

providing a signal detector that detects the emitted signal;

coupling a first mixer to said excitation signal generator, said mixer mixes the emitted signal with the driving/reference signal to produce the processor reference signal,

coupling a second mixer to said mixing signal generator, said mixer mixes the emitted signal with the mixing signal to produce the data signal; and

coupling a processor to said first mixer and said second mixer, said processor extracts the fluorescence lifetime and fluorescence spectrum of the emitted signal from the comparison of the processor reference signal with the data signal using a chemometric analysis.

17. The method of claim **16** wherein the driving/reference signal and the mixing signal vary by an adjustable offset frequency.

18. The method of claim **16** wherein said chemometric analysis extracts the fluorescence lifetime of the emitted signal from the phase difference between the processor reference signal and the data signal.

19. The method of claim **16** wherein said chemometric analysis extracts the fluorescence spectrum of the emitted signal from the amplitude difference between the processor reference signal and the data signal.

20. The method of claim **16** wherein said chemometric analysis further comprises a converging iterative solution.

21. A program storage device readable by a computer, tangibly embodying a program of instructions executable by the computer to perform method steps for a method for measuring the fluorescence lifetime and the fluorescence spectrum, comprising the following method steps:

generating a driving/reference signal and modulating the amplitude and/or the frequency of the driving/reference signal over time;

generating a mixing signal and modulating the amplitude and/or the frequency of the mixing signal over time;

generating an excitation signal from the driving/reference signal;

detecting the emitted signal;

mixing the emitted signal with the driving/reference signal and producing the processor reference signal;

mixing the emitted signal with the mixing signal producing the data signal; and

extracting the fluorescence lifetime and fluorescence spectrum of the emitted signal from the comparison of the processor reference signal with the data signal to measure using a chemometric analysis.

22. The program storage device of claim **21** wherein the driving/reference signal and the mixing signal vary by an adjustable offset frequency.

23. The program storage device of claim **21** wherein said chemometric analysis extracts the fluorescence lifetime of the emitted signal from the phase difference between the processor reference signal and the data signal.

24. The program storage device of claim **21** wherein said chemometric analysis extracts the fluorescence spectrum of the emitted signal from the amplitude difference between the processor reference signal and the data signal.

25. The program storage device of claim **21** wherein said chemometric analysis further comprises a converging iterative solution.

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